AMENDED CLAIMS

[received by the International Bureau on 2 February 1998 (02.02.98); original claim 2 amended; remaining claims unchanged (5 pages)]

WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding a monomer of a calcium-activated potassium channel, said monomer:

i. having a calculated molecular weight of between 40 and 80 kDa;

ii. having a unit conductance of between 2 and 60 pS when the monomer is in the functional polymeric form of a potassium channel and is expressed in a *Xenopus* oocyte; and;

iii. specifically binding to antibodies generated against SEQ ID NOS:30 or 42.

2. An isolated nucleic acid encoding at least 15 contiguous amino acids from a calcium-activated potassium channel protein, said protein having a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, SEQ ID NO:47 and conservatively modified variants thereof, with the provisio that the contiguous amino acids do not consist of a glutamine repeat amino acid sequence.

3. The isolated nucleic acid of claim 2 wherein said nucleic acid encodes a calcium activated potassium channel protein having a conductance of between 2 and 60 pS and a molecular weight of between 40 and 80 kilodaltons and

wherein said nucleic acid either

i. selectively hybridizes under moderate stringency hybridization conditions to a sequence selected from the group consisting of SEQ ID NOS:13, 14, 15, 16, 21, 22, 31, 44, and 48, or

ii. encodes a protein which could be encoded by a nucleic acid that selectively hybridizes under moderate stringency hybridization conditions to a sequence selected from the group consisting of SEQ ID NOS:13, 14, 15, 16, 21, 22, 31, 44, and 48.

4. The isolated nucleic acid of claim 1, wherein said nucleic acid encodes a protein having a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, and SEQ ID NO:47.

5. The isolated nucleic acid of claim 1, wherein said nucleic acid encodes a protein having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:43.

6. An isolated nucleic acid of claim 1 wherein the sequence is identical to a naturally occurring sequence.

7. An isolated nucleic acid of claim 1 having the sequence depicted in SEQ ID NO:31.

8. An isolated nucleic acid of claim 1 encoding at least 15 contiguous amino acids of a monomer of an intermediate conductance, calcium-activated potassium channel, said monomer:

i. having a calculated molecular weight of about 42 to 52 kDa;

ii. having units conductance of between 30 and 60 pS in the inward direction when the monomer is in the functional polymeric form of a potassium channel and is expressed in a *Xenopus* oocyte; and,

iii. specifically binding to a polyclonal antibody generated against SEQ ID NO:32.

9. An isolated nucleic acid of claim 8 wherein the sequence is identical to a naturally occurring sequence.

10. An isolated nucleic acid of claim 6 encoding any 8 contiguous amino acids from the following sequence:

VRGPPCVQDLGAPLTSPQPWPGFLGQGEAL (SEQ ID NO:33).

11. An isolated nucleic acid sequence of at least 20 nucleotides in length which specifically hybridizes, under stringent conditions, to a nucleic acid

encoding an intermediate calcium-activated potassium channel protein, said protein selected from the group consisting of SEQ ID NO:32.

12. An isolated calcium-activated potassium channel protein having at least 15 contiguous amino acids from a sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, SEQ ID NO:47 and conservatively modified variants thereof, wherein said variants specifically react, under immunologically reactive conditions, with an antibody reactive to a protein selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, and SEQ ID NO:47.

13. The isolated calcium-activated potassium channel protein of claim 12, wherein said protein when expressed in a *Xenopus* oocyte leads to formation of an calcium-activated potassium channel having a conductance of between 2 and 80 pS and a molecular weight of between 40 and 80 kilodaltons.

14. The isolated calcium-activated potassium channel protein of claim 12, wherein said protein has a sequence shown in SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32 or SEQ ID NO:47.

15. The isolated calcium-activated potassium channel protein of claim 12, wherein said protein has a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:43.

16. An isolated protein of claim 12 comprising at least 15 contiguous amino acids from a monomer of a calcium-activated potassium channel protein having

i. having a calculated molecular weight of about 42 to about 52 kDa;

ii. having units conductance of between 30 and 60 pS in the inward direction when the monomer is in the functional polymeric form of a potassium channel and is expressed in a *Xenopus* oocyte; and;

iii. specifically binding to a polyclonal antibody generated against SEQ ID NO:32.

17. An isolated protein of claim 16 having an amino acid sequence identical to a naturally occurring sequence.

18. An isolated protein of claim 16 having the sequence depicted in SEQ ID NO:32.

19. An isolated nucleic acid of claim 16 encoding any 8 contiguous amino acids from the following sequence:

VRGPPCVQDLGAPLTSPQPWPGFLGQGEAL (SEQ ID NO:33).

20. An isolated intermediate conductance calcium-activated potassium channel protein encoded by a nucleic acid a portion of which when amplified by primer pairs produces an amplified fragment which selectively hybridizes, under stringent hybridization conditions to SEQ ID NO:31 wherein said primer pairs are selected from the group consisting of:

5' GCCGTGCGTGCAGGATTTAGG 3' (SEQ ID NO:34)
5' CCAGAGGCCAAGCGTGAGGCC 3' (SEQ ID NO:35);
5' TCCAAGATGCACATGATCCTG 3' (SEQ ID NO:36); and,
5' GGACTGCTGGCTGGGTTCTGG 3' (SEQ ID NO:37).

21. An antibody specifically reactive, under immunologically reactive conditions, to a calcium-activated potassium channel protein, said protein having a sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, and SEQ ID NO:47.

22. An antibody of claim 21, wherein said antibody is specifically reactive to the protein selected from the group consisting of SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, and SEQ ID NO:47.

23. An antibody of claim 21, wherein said protein has a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:43.

24. The antibody of claim 21, wherein said antibody is a monoclonal antibody.

25. The antibody of claim 24, wherein said monoclonal antibody is specifically reactive to a protein selected from the group consisting of SEQ ID NO:1, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, and SEQ ID NO:47.

26. An expression vector comprising a nucleic acid encoding a calcium-activated potassium channel protein, said channel protein having a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO: 43, and SEQ ID NO: 47, and conservatively modified variants thereof, wherein said conservatively modified variant is a protein which when expressed in an oocyte leads to formation of a calcium-activated potassium channel having a conductance of between 2 and 80 pS, a molecular weight of between 40 and 80 kilodaltons, and specifically reacts, under immunologically reactive conditions, with an antibody reactive to the channel protein selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, and SEQ ID NO:47.

27. A host cell transfected with the vector of claim 26.

28. An isolated nucleic acid sequence of at least 20 nucleotides in length which specifically hybridizes, under stringent conditions, to a nucleic acid encoding a calcium-activated potassium channel protein, said protein selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, and SEQ ID NO:47.

29. A method for detecting the presence of a calcium-activated potassium channel protein in a biological sample, said method comprising:

(a) contacting said biological sample with an antibody, wherein said antibody specifically reacts, under immunologically reactive conditions, to the channel protein having a sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, and SEQ ID NO:47;

(b) allowing said antibody to bind to said protein under immunologically reactive conditions, wherein detection of said bound antibody indicates the presence of said channel protein.

30. A method for detecting the presence, in a biological sample, of a nucleic acid sequence encoding a calcium-activated potassium channel protein of at least 25 amino acids in length, said method comprising:

(a) contacting said sample, under stringent hybridization conditions, with a nucleic acid probe comprising a nucleic acid segment that selectively hybridizes to a nucleic acid encoding said channel protein having a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3,SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, and SEQ ID NO:47;

(b) allowing said nucleic acid encoding the channel protein to selectively hybridize to said probe to form a hybridization complex, wherein detection of said hybridization complex is an indication of the presence of said nucleic acid sequence in said sample.

31. An isolated calcium-activated potassium channel protein encoded by a nucleic acid amplified by primers which selectively hybridize, under stringent hybridization conditions, to the same sequence as primers selected from the group consisting of:

for hSK1

ATGCCGGGTCCCCGGGCGGCCTGC (SEQ ID NO:5)

TCACCCGCAGTCCGAGGGGGCCAC (SEQ ID NO:6)

for rSK2

ATGAGCAGCTGCAGGTACAACGGG (SEQ ID NO:7)

CTAGCTACTCTCAGATGAAGTTGG (SEQ ID NO:8)

for rSK3

ATGAGCTCCTGCAAATACAGCGGT (SEQ ID NO:9)

TTAGCAACTCTGTGAACTTG (SEQ ID NO:10)

for rSK1

TCAGGGAAGCCCCCGACCGTCAGT (SEQ ID NO:11)

TCACCCACAGTCTGATGCCGTGGT (SEQ ID NO:12)

for hSK2

ATGAGCAGCTGCAGGTACAACG (SEQ ID NO:23)

CTAGCTACTCTCTGATGAAGTTG (SEQ ID NO:24)

for hSK3

ATGAGCTCCTGCAAGTATAGC (SEQ ID NO:25)

TTAGCAACTGCTTGAACTTGTG (SEQ ID NO:26)

for hIK1

GCCGTGCGTGCAGGATTTAGG (SEQ ID NO:34)

CCAGAGGCCAAGCGTGAGGCC (SEQ ID NO:35);

and,

for hIK1

TCCAAGATGCACATGATCCTG (SEQ ID NO:36)

GGACTGCTGGCTGGGTTCTGG (SEQ ID NO:37).

32. A method of identifying a compound which increases or decreases the potassium ion flux through a calcium-activated potassium channel, the steps of:

a) contacting said compound with a eukaryotic host cell or cell membrane in which has been expressed a calcium-activated potassium channel protein having a sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, and SEQ ID NO:47 conservatively modified variants thereof, wherein said conservatively modified variants specifically bind to antibodies specifically reactive with an antigen having an amino acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, and SEQ ID NO:47, have a conductance of between 2 and 80 pS, and a molecular weight between 40 and 80 kilodaltons; and

b) determining the functional effect of the compound upon the cell or cell membrane expressing said channel.

33. The method of claim 32, wherein the increased or decreased flux of potassium ions is determined by measuring the electrical current across the cell membrane of said eukaryotic host cell.

34. The method of claim 32, where said channel protein has a sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, and SEQ ID NO:47.

35. The method of claim 32, wherein said channel protein is recombinant.

36. The isolated nucleic acid of claim 1, said nucleic acid having a sequence selected from the group consisting of: SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:31, SEQ ID NO:44, and SEQ ID NO:48.

37. An isolated eukaryotic nucleic acid encoding a calcium-activated channel protein of at least 400 amino acid residues in length, wherein said channel protein comprises an amino acid sequence having at least 60% similarity over at least the length of a core region of a protein selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, and SEQ ID NO:47, and wherein said channel protein has a conductance of between 2 and 80 pS.

38. The isolated nucleic acid of claim 37, wherein said channel protein has at least 90% sequence similarity over a comparison window of at least 20 contiguous amino acid residues within said core region.

39. A vector comprising the isolated nucleic acid of claim 37.

40. A host cell transfected with the vector of claim 39.

41. A method of making a calcium-activated potassium channel protein, comprising culturing the host cell of claim 40 under conditions permitting expression of said nucleic acid encoding said channel protein.

42. A method of making a calcium-activated potassium channel protein, comprising culturing the host cell of claim 27 under conditions permitting expression of said nucleic acid encoding said channel protein.

43. A method of identifying a compound that increases or decreases the potassium ion flux through a calcium-activated potassium channel, the steps of:

a) contacting said compound with a host cell or cell membrane in which has been expressed a calcium-activated potassium channel protein of at least 400 amino acid residues in length, wherein said SK channel protein has an amino acid sequence having at least 60% similarity over the length of the core region of a protein selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:32, SEQ ID NO:43, and SEQ ID NO:47, and wherein said channel protein has a conductance of between 2 and 80 pS; and

b) determining the functional effect of the compound upon the cell or cell membrane expressing the channel.

44. The method of claim 30, wherein said stringent hybridization conditions are moderate stringency hybridization conditions.

45. The method of claim 44, wherein said nucleic acid sequence encodes a calcium-activated potassium channel protein of at least 400 amino acid residues in length and having a conductance of between 2 and 80 pS.

46. The method of claim 45, wherein said nucleic acid probe comprises at least 250 contiguous nucleotides encoding a subsequence within said channel protein core region.

47. The protein encoded by the isolated nucleic acid of claim 37.

48. The method of claim 43, wherein the increased or decreased flux of potassium ions is determined by measuring the electrical current across the cell membrane of said eukaryotic host cell.

49. In a computer system, a method of screening for mutations of SK and IK genes, the method comprising the steps of:

(i) receiving input of a first nucleic acid sequence encoding a calcium-activated channel protein having a sequence selected from the group consisting of SEQ ID NOS:1, 2, 3, 4, 19, 20, 32, 43, 47 and conservatively modified versions thereof;

(ii) comparing the first nucleic acid sequence with a second nucleic acid sequence having substantial identity to the first nucleic acid sequence; and

(iii) identifying nucleotide differences between the first and second nucleic acid sequences.

50. The method of claim 49, wherein the second nucleic acid sequence is associated with a disease state.

51. In a computer system, a method for identifying a three-dimensional structure of SK and IK proteins, the method comprising the steps of:

(i) receiving input of an amino acid sequence of a calcium-activated channel protein or a nucleotide sequence of a gene encoding the protein, the protein having an amino acid sequence selected from the group consisting of SEQ ID NOS:1, 2, 3, 4, 19, 20, 32, 43, 47, and conservatively modified versions thereof; and

(ii) generating a three-dimensional structure of the protein encoded by the amino acid sequence.

52. The method of claim 51, wherein said amino acid sequence is a primary structure and wherein said generating step includes the steps of forming a secondary structure from said primary structure using energy terms encoded by the primary structure and forming a tertiary structure from said secondary structure using energy terms encoded by said secondary structure.

53. The method of claim 51, wherein said generating step includes the step of forming a quaternary structure from said tertiary structure using anisotropy terms encoded by the tertiary structure.

54. The method of claim 52, wherein said generating step further includes the step of forming a quaternary structure from said tertiary structure using anisotropy terms encoded by the tertiary structure.

55. The method of claim 50, further comprising the step of identifying regions of the three-dimensional structure of the protein that bind to ligands and using the regions to identify ligands that bind to the protein.

Add B1